

## Description

[Novel topical skin care and nutraceutical applications of Glabridin or extracts containing a defined amount (4–90%) of Glabridin ]

### CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application is a continuation-in-part of pending US Patent Application # 10065995 filed on December 9, 2002, for a Commercial Process for Isolation and Purification of Glabridin with High Tyrosinase Inhibitory Activity and its Cosmetic Compositions and Methods Of Use, the disclosure of which is hereby incorporated by reference.

### BACKGROUND OF INVENTION

#### [FIELD OF INVENTION]

[0002] The present invention relates to additional cosmetic, particularly skin care, applications and compositions with glabridin. More particularly, in addition to the invention described in the parent patent application, the current in-

vention discloses the uses of Glabridin as such, or in the form of licorice extract containing a 4–90% glabridin as metalloprotease and hyaluronidase inhibiting component in formulations for topical or oral use. Glabridin is useful in anti-wrinkle and anti-aging products, providing elasticity, firmness, tone and texture to the skin, ameliorating fine lines and crows feet in under eye preparations, and prevent skin and hair damage due to UV rays, inflammation and itch, diaper rashes in baby products, as massage or toning oils or emulsion for babies.

[0003] Orally, the said extract could be used in maintaining and alleviating conditions of arthritis, joint immobility, osteoarthritis and conditions manifested due to increased activity of elastase, collagenase and hyaluronidase enzymes.

#### **[DESCRIPTION OF PRIOR ART]**

[0004] Isoflavones are a larger and distinctive subclass of flavonoids. These compounds possess a 3-phenyl chromane skeleton that is biogenetically derived by rearrangement of the flavonoid 2-phenyl chromane system (1,2 diaryl rearrangement). Isoflavonoids are almost entirely distributed to the subfamily Papilionaceae of Leguminosae family. Several flavonoids are potent inhibitors of lipoxge-

nase or cyclooxygenase or both. These properties explain their antiinflammatory and antiallergenic activity.

Glabridin an isoflavan found in licorice extracts is reported to have anti-inflammatory, antioxidant and tyrosinase inhibitory properties (Yokota, T. et al. *Pigment Cell Res.* 11(6):355,361, 1998; Vaya, J. *Free Rad. Biol. Med.* 23(2):302–313, 1997). Methods to isolate isoflavans and other tyrosinase inhibitors have been described in literature (Mitscher, L. et al. *J. Nat. Prod.* 43(2):259–269, 1980; Shiota, S. et al. *Biol. Pharm. Bull.* 17(2):266–269, 1994; Saitoh, T. et al. *Chem. Pharm. Bull.* 24(4):742–755) UV induced oxidative stress leading to unbridled production of free radicals is documented to augment the activity of enzymes such as elastase, collagenase and hyaluronidase. This causes premature degradation and digestion of the key structural components elastin and collagen of the dermis.

[0005] The long term consequence of the prolonged activation of these enzymes is what is believed to lead to premature skin aging. The formation of fine lines and wrinkles or the appearance of slacking skin, is not apparently caused by direct photochemical free radical controlled reactions, but rather by the activation at the genomic level of the skin's

own enzymes as a consequence of the of immune system-mediated inflammatory responses. Thus the intervention at the stage of arresting the enzyme synthesis and activity (at the genomic and the proteomic level), is essential in controlling the progress of premature skin aging, loss of elasticity, tone, loss of suppleness, inflammation , itch or irritation. (Maes DH, Marenus KD, Textbook of Cosmetic Dermatology,Ed., Robert Baran and Howard Maibach, Pbs: Martin Dunitz Ltd.,469–485, 1998). Prior art teaches that high activity of collagenase and elastase in the Synovial fluids (SF) of patients with rheumatoid arthritis (RA), which is about 30 times higher than that found in the SF of patients with osteoarthritis (OA). These findings underline the synergic action of these enzymes in the pathogenesis of joint damage. RA patients also exhibit higher levels of glutathione reductase, which is important for the detoxification pathway of oxygen free radicals. However, compared with findings for collagenase and elastase, the increase in glutathione reductase is only three times higher than level found in the SF of OA patients. (Bazzichi L, Ciompi ML, Betti L, Rossi A, Melchiorre D, Fiorini M, Giannaccini G, Lucacchini A., Clin Exp Rheumatol. 2002 Nov-Dec;20(6):761–6. Impaired glutathione reductase activity

and levels of collagenase and elastase in synovial fluid in rheumatoid arthritis).

[0006] Diaper dermatitis generally called "diaper rash" manifests itself as primary irritation dermatitis brought about by physical irritation from the diaper surface, chemical irritation from stools and urine and clinically take the form of erythema, papules, edema accompanied by itching. Proteolytic and lipolytic enzymes from faeces especially elastase causes increased vasodilation, increase in transepidermal water loss and skin pH, with higher irritation potential. Prior art suggest that the possible etiologic role of proteases in perianal, circumstomal or diaper dermatitis. (Andersen PH et al., Faecal enzymes: invivo human skin irritation. Contact Dermatitis, 1994, 30(3) 152-8)

[0007] Implantation of the embryo into the endometrium is a highly regulated event that is critical for establishment of pregnancy. Molecules involved in this process provide potential targets for post-coital contraception. Administration of MMP (Matrix Metalloprotease) inhibitors in animals during early pregnancy retards decidual development. (Rechtman MP, Zhang J, Salamonsen LA., J Reprod Fertil. 1999 Sep;117(1):169-77). Effect of inhibition of matrix metalloproteinases on endometrial decidualization and

implantation in mated rats.) Progesterone, a orally used contraceptive, is a key suppressor of the activity of MMPs in the endometrium. In cultured explants from untreated women, progesterone abrogates the expression of procollagenase-1 and the activation of progelatinase B. It also inhibits the expression of progelatinases A and B and further decreases the activities of all of these MMPs by stimulating the production of TIMP-1 (The Journal of Clinical Endocrinology & Metabolism Vol. 85, No. 12 4827-4834. Christine Galant, et al., Temporal and Spatial Association of Matrix Metalloproteinases with Focal Endometrial Breakdown and Bleeding upon Progestin-Only Contraception) All of the prior art as described in the main patent application use Licorice extract.

[0008] Surprisingly, none of the prior art describe the use of glabridin, isolated from licorice roots or extracts containing a defined amount of Glabridin for elastase inhibition or collagenase or hyaluronidase inhibition and its use as anti-wrinkle, anti-aging, anti-itch or orally in the treatment of arthritis or baby care diaper rashes or in conditions of dry skin syndrome or use in preventing photoaging. In this invention, we provide evidence that glabridin from licorice extract or licorice extract containing from 4%

to 90% of glabridin inhibit MMPs and hyaluronidase enzymes in skin. We claim the use of these extracts for skin and nutraceutical applications.

## **SUMMARY OF INVENTION**

- [0009] The present invention discloses a surprising new finding that lipophilic licorice extract containing Glabridin at various purity (4%, 40% and 80%) is an excellent elastase, collagenase and hyaluronidase inhibitor. The extract containing glabridin or glabridin alone or in combination with other cosmetic actives can be used to fight wrinkles, fine lines, premature aging, photoaging, skin tone, itch and orally for the treatment of arthritis, osteoarthritis, inflammation, tumors and as contraceptives. Compositions containing glabridin and their use in skin care are described.
- [0010] The object of the present invention is to provide cosmetic compositions having metalloprotease inhibition.
- [0011] It is another object of the present invention to provide compositions that have number of benefits in connection with skin care and prevent the damage to the keratinous tissue.
- [0012] The compositions of this invention may be in the form of gel, lotion, anhydrous sticks, oil based sprays, oil-in-water or water-in-oil emulsion and any other oral

dosage form.

[0013] These and other objects of the present invention are achieved by a cosmetic composition comprising essentially of Glabridin or an extract containing Glabridin (4%, 40% or 80%), as elastase and collagenase inhibitor along with one or more antioxidants, sunscreens, emulsifiers, preservatives, additional anti-wrinkle agents, thickeners and fragrances.

#### **BRIEF DESCRIPTION OF DRAWINGS**

[0014] Figure 1: Effect of content of glabridin on IC50 of Collagenase

[0015] Figure 2: Effect of content of glabridin on IC50 of Elastase

[0016] Figure 3: Effect of content of glabridin on IC50 of Hyaluronidase

#### **DETAILED DESCRIPTION**

[0017] The present invention, a continuation-in-part of the parent patent application, includes a skin care composition containing from about 0.001% to about 10% preferably from 0.1 to 3% most preferably from 0.1 to 0.5% by weight of a composition of purified glabridin containing 4, 20, 40 or 90% glabridin by weight and a cosmetically acceptable vehicle. The purity of glabridin in licorice ex-



tracts used in the compositions of the present invention, is selected at optimal levels to support the multiple functions of tyrosinase inhibition, metalloprotease inhibition, antioxidant effects and UV protective effects.

[0018] Glabridin of varying strengths have been studied for their inhibition of elastase, collagenase and hyaluronidase to ascertain its use as anti-aging, anti-wrinkle and anti-itch properties .

[0019] Further, in accordance with the present invention, there have been disclosed, cosmetic compositions, preferable in the form of oil-in-water emulsion. The composition contains glabridin with or without one or more of the following tyrosinase inhibitors: Tetrahydrocurcumin, Tetrahydrodemethoxycurcumin, Tetrahydrobisdemethoxycurcumin curcumin, demethoxycurcumin, bisdemethoxycurcumin, ellagic acid, soy isoflavones. The composition may also include one or more triterpenic acids as anti-wrinkle component, antioxidants, sunscreens, emulsifiers, preservatives and thickeners.

[0020] The compositions of the present invention offer a number of benefits as anti-wrinkle, anti-aging, anti-itch , baby care diaper rashes or orally in the treatment of arthritis or in conditions of dry skin syndrome or use in preventing

photoaging .

[0021] *Example 1 Concentration dependent functional properties of glabridin a) Method for anti-collagenase activity* . The assays were done using the EnzChek collagenase assay kit. The substrate was DQ gelatin (from pig skin) (source Enzy-Check kit). DQ gelatin is a fluorescein conjugated-gelatin labeled with fluorescein. The fluorescence is quenched in the presence of inhibitor. The reduction in fluorescence intensity was measured in a microplate reader / Fluostar Optima (emission at 485nm and excitation at 520nm.)  
Procedure : Aliquots of the enzyme solution (100µl of 0.4U/ml collagenase-Type IV from Clostridium histolyticum) and different concentrations of the material in DMSO (80µl) were preincubated in a microplate for 10 minutes. After preincubation, 20µl of the substrate DQ gelatin (12.5µg/ml) was added and the fluorescence intensity was measured after 30 minutes. Enzyme activity with DMSO and controls were also taken. The final concentration of DMSO in the reaction mixture was 3%, which did not show any significant effect on the enzyme activity. Calculations: The percentage inhibition is calculated as follows: –

$$\% \text{ Inhibition} = \frac{(B-BC)-(T-C)}{(B-BC)} \times 100$$

B – Fluorescence in the presence of enzyme  
 BC – Fluorescence in the absence of enzyme activity  
 T – Fluorescence of enzyme activity in the presence of inhibitor  
 C – Fluorescence of the inhibitor alone

[0022] *b) Method for anti-elastase activity* . The assays were done using the EnzChek elastase assay kit. The substrate is DQ elastin from soluble bovine neck ligament. DQ elastin is labeled with BODIPY FL dye such that the conjugate's fluorescence is quenched in the presence of inhibitor. The reduction in fluorescence intensity was measured in a microplatereader/ Fluostar Optima (emission at 485nm and excitation at 520nm.) Procedure: Aliquots of the enzyme solution (100µl of 0.5U/ml of elastase from pig pancreas) and different concentrations of the material in DMSO (50µl) were preincubated in a microplate for 10 minutes. After preincubation, 50µl of the substrate DQ elastin (25µg/ml) ( EnzChek ) was added and the fluorescence intensity was measured after 30 minutes. Enzyme activity with DMSO and controls were also taken. The final concentration of DMSO in the reaction mixture was 3%, which did not show any significant effect on the enzyme activity.

[0023] Calculations: The percentage inhibition is calculated as follows: –

$$\% \text{ Inhibition} = \frac{(B-BC)-(T-C)}{(B-BC)} \times 100$$

B – Fluorescence in the presence of enzyme

BC – Fluorescence in the absence of enzyme activity

T – Fluorescence of enzyme activity in the presence of inhibitor

C – Fluorescence of the inhibitor alone

[0024] *c) Method for Anti- hyaluronidase activity* Ref:– 1) Sigma method for the enzymatic assay of hyaluronidase 2) Faizyme Assay procedure Ref No: FGAP045 hyaluronidase 3) Jwu–Sheng Tung, George.E. Mark and Gregory. F. Hollis. Dept of Cellular and Molecular Biology, Merck Research Laboratories, New Jersey 07065: A Microplate assay for Hyaluronidase inhibitors.

[0025] **Materials and Methods** Hyaluronic acid (from human umbilical cord), hyaluronidase (H 3884 from bovine testis), cetyl pyridinium chloride and the other reagents were obtained from Sigma. Hyaluronic acid (HA) was dissolved in 300mM sodium phosphate buffer pH 5.35. Agarose was dissolved in the same buffer and maintained at 55°C before use. HA solution was preheated to 55°C and mixed with agarose to give a final concentration of 0.5 mg/ml of HA and 0.8% of agarose. Warm HA–agarose mixture (100µl) was dispensed into each well of a microplate and allowed to set. For screening of inhibitors, in another microplate, each well was filled with 100µl of the HA ase (10

units / test in 10 mM sodium phosphate buffer with 77mM sodium chloride pH 7.0) and 100µl of the inhibitor dissolved in DMSO were preincubated at 37°C for 10 minutes. A final concentration of 1% DMSO was used which did not have any effect on the enzyme activity. Enzyme activity with DMSO and controls were also kept. After preincubation 100µl from these samples were removed and overlaid onto the pre-set HA/agarose gels wells in triplicates and were incubated at 37°C for 45 minutes. After incubation enzyme samples were removed, and each well was filled with 100µl of 10% aqueous cetyl pyridinium chloride. The absorbance was measured in a microplate reader/ Fluostar Optima at 600 nm after 10 minutes, at room temperature. A standard plot was also done with the same procedure using different concentrations of hyaluronic acid and a linearity of  $R^2 = 0.99$  was observed between 0.1 to 0.7 mg/ml of HA concentration.

[0026] Calculations: The results are expressed as IC 50 values, the concentration at which the compound inhibits half the original hyaluronidase activity. The percentage of inhibition is calculated as follows:

$$\% \text{ Inhibition} = \frac{((EC-EA)-(EC-[T-TC])) \times 100}{(EC-EA)}$$

where EC – Absorbance in the absence of enzyme and inhibitor

EA – Absorbance in the presence of enzyme activity

T – Absorbance of enzyme activity in the presence of inhibitor

TC – Absorbance of the inhibitor alone

[0027] **Results :** The findings indicate that while Licorice extract containing 90% glabridin has higher collagenase inhibition, Licorice extract containing 40% Glabridin is better as elastase and hyaluronidase Inhibitor

Sample	Collagenase	Elastase	Hyaluronidase
	<b>IC50</b>	<b>IC50</b>	<b>IC50</b>
Glabridin-0.2%	>1000	>1000	>1000
Glabridin-4%	12.5µg	10µg	12.5µg
Glabridin-40%	25µg	4.8µg	15µg
Glabridin-90%	9µg	11µg	25µg
Ursolic acid 40%	18µg	26µg	19µg
Ursolic acid 90%	6.25µg	15µg	10µg

[0028] Licorice extract containing 4% Glabridin has a disadvantage of being dark reddish brown colored which invariably colors the product. This can be overcome by use of 40% Glabridin which is pale yellow to off white in color.

[0029] In order to formulate an effective cosmetic product, the individual ingredients have to be solublized in cosmetically acceptable solvents. The following table indicates the ease of solubility of glabridin and the licorice extract in contrast to Ursolic acid, a terpenoid compound used in

cosmetics for similar claims. The data clearly indicate that glabridin is much more easily soluble than Ursolic acid. This data indicate the ease of the use of glabridin for cosmetic formulations.

Sample Assay/Purity	Licorice Extract 4.0% glabridin	Licorice Extract 40% glabridin	URSOLIC ACID 90%
Water	Insoluble	Insoluble	Insoluble
Ethanol	8 %*	22.60 %	< 0.1 %
Glycerin	<0.1 %	<0.1 %	< 0.1 %
Propylene Glycol	1.5 %*	3.00 %	< 0.1 %
Butylene Glycol	2.5 %*	3.00 %	< 0.1 %
Hexylene Glycol	2.5 %	3.00 %	< 0.1 %
Polyethylene Glycol 300	2 %	2.80 %	Insoluble
Paraffin oil	< 0.1 %	< 0.1 %	< 0.1 %
Methyl soyate	< 0.1 %	2.70 %	< 0.1 %
Triethyl Citrate	2.1 %	4.00 %	< 0.1 %
Isopropyl Myristate	< 0.3 %	3.50 %	< 0.1 %
Methyl Benzoate	1.25 %	4 %	< 0.1 %
Soyabean oil	<0.1 %	1.30 %	< 0.1 %
N-methyl pyrrolidone	18 %	21.00 %	4.10 %
Coconut oil- Fraction A (Lauric acid-rich)	<0.1 %	2.50 %	< 0.1 %

[0030] While the invention has been described with particular reference to certain embodiments thereof, it should be understood that changes and modifications may be made which are within the skill of the art without affecting the spirit of invention as mentioned under the claims.